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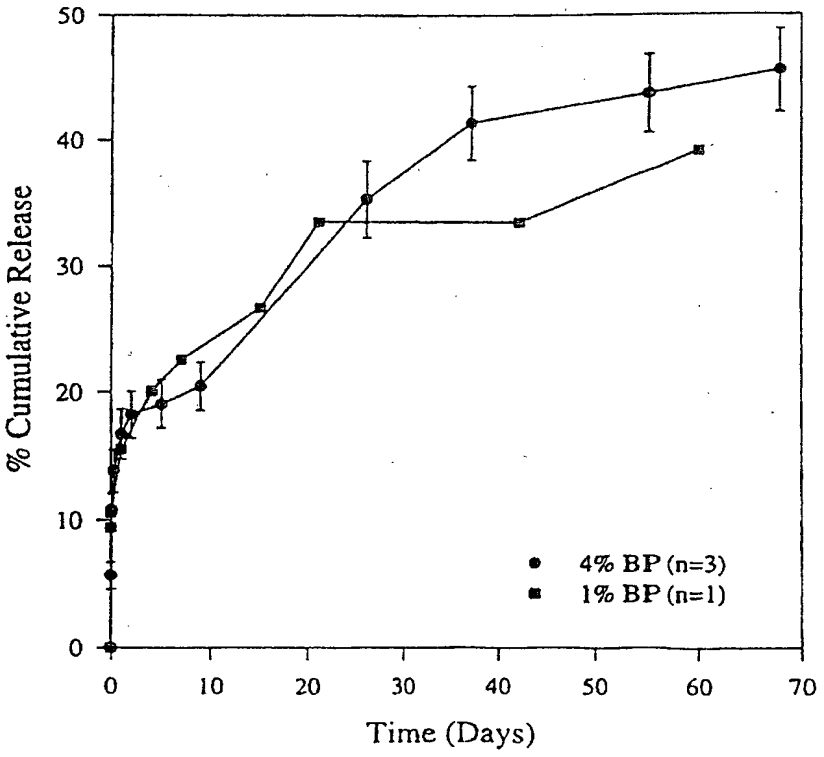
(54) Title: COMPOSITION AND DEVICE FOR IN VIVO CARTILAGE REPAIR

(57) Abstract

The composition as described serves for in vivo cartilage repair. It basically consists of a naturally derived osteoinductive and/or chondroinductive mixture of factors (e.g., derived from bone) or of a synthetic mimic of such a mixture combined with a nanosphere delivery system. A preferred mixture of factors is the combination of factors isolated from bone, known as BP and described by Poser and Benedict (WO 95/13767). The nanosphere delivery system consists of nanospheres defined as polymer particles of less than 1000 nm in diameter (whereby the majority of particles preferably ranges between 200–400 nm) in which nanospheres the combination of factors is encapsulated. The nanospheres are loaded with the mixture of factors in a weight ratio of 0.001 to 17 % (w/w), preferably of 1 to 4 % (w/w) and have a release profile with an initial burst of 10 to 20 % of the total load over the first 24 hours and a long time release of at least 0.1 per day during at least seven following days. The nanospheres are composed of e.g. ((D,L)–lactic acid/glycolic acid)–copolymer (PLGA). The loaded nanospheres are e.g. made by phase inversion. The composition is advantageously utilized as a device comprising any biodegradable matrix in which the nanospheres loaded with the factor combination is contained.



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COMPOSITION AND DEVICE FOR IN VIVO CARTILAGEREPAIR

Background of the Invention:

Articular cartilage, an avascular tissue found at the ends of articulating bones, has no natural capacity to heal. During normal cartilage ontogeny, mesenchymal stem cells condense to form areas of high density and proceed through a series of developmental stages that ends in the mature chondrocyte. The final hyaline cartilage tissue contains only chondrocytes that are surrounded by a matrix composed of type II collagen, sulfated proteoglycans, and additional proteins. The matrix is heterogenous in structure and consists of three morphologically distinct zones: superficial, intermediate, and deep. Zones differ among collagen and proteoglycan distribution, calcification, orientation of collagen fibrils, and the positioning and alignment of chondrocytes (Archer et al., J. Anat. 189(1): 23-35, 1996; Morrison et al., J. Anat. 189(1): 9-22 1996, Mow et al., Biomaterials 13(2): 67-97, 1992). These properties provide the unique mechanical and physical parameters to hyaline cartilage tissue.

In 1965, a demineralized extraction from bovine long bones was found to induce endochondral bone formation in the rat subcutaneous assay (Urist Science 150: 893-899, 1965). Seven individual factors, termed Bone Morphogenetic Proteins (BMPs), were isolated to homogeneity and, because of significant sequence homology, classified as members of the TGFß super-family of proteins (Wozney, et al., Science 242: 1528-34, 1988; Wang et al., Proc. Nat. Acad. Sci. 87: 2220-2224, 1990). These individual, recombinantly-produced factors also induce ectopic bone formation in the rat model (Luyten et al., J. Biol. Chem. 264: 13377-80, 1989; Celeste et al., Proc. Nat. Acad. Sci. 87: 9843-50, 1990). In addition, in vitro tests have demonstrated that both BMP-2 and TGFß-1 induce mesenchymal stem cells to form cartilage (Denker, et al., Differentiation 59(1): 25-34, 1995; Denker et al., 41st Ann. Orthop. Res. Society 465: 1995). Both BMP-7 and BMP-2 have been shown to enhance matrix production of chondrocytes in vitro (Flechtenmacher J. Arthritis Rheum. 39(11): 1896-904, 1996: Sailor et al., J. Orthop. Res. 14: 937-945, 1996). From these data we can conclude that not only are the BMPs important regulators of osteogenesis, but that they also play crucial roles during chondrogenic development in vitro.

A partially-purified protein mixture from bovine long bones, termed BP (Bone Protein), also induces cartilage and bone formation in the rat subcutaneous assay (Poser and Benedict, WO95/13767). BP in combination with calcium carbonate promotes bone formation in the body. In vitro, BP induces mesenchymal stem cells to differentiate specifically to the cartilage lineage, in high yields, and to late stages of maturation (Atkinson et al., J. Cellular Biochem. 65: 325-339, 1997).

The molecular mechanism for cartilage and bone formation has been partially elucidated. Both BMP and TGFß molecules bind to cell surface receptors (the BMP/TGFß receptors), which initiates a cascade of signals to the nucleus that promotes proliferation, differentiation to cartilage, and/or differentiation to bone (Massague Cell 85: 947-950, 1996).

In 1984, Urist described a substantially pure, but not recombinant BMP, combined with a biodegradable polylactic acid polymer delivery system for bone repair (US-4,563,489). This system blends together equal quantities of BMP and polylactic acid (PLA) powder (100 µg of each) and decreases the amount of BMP required to promote bone repair.

Hunziker (US-5,368,858; US-5,206,023) describes a cartilage repair composition consisting of a biodegradable matrix, a proliferation and/or chemotactic agent, and a transforming factor. A two stage approach is used where each component has a specific function over time. First, a specific concentration of proliferation/chemotactic agent fills the defect with repair cells. Secondly, a larger transforming factor concentration transforms repair cells into chondrocytes. Thereby the proliferation agent and the transforming agent may both be TGFß differing in concentration only. In addition, the patent discloses a liposome encapsulation method for delivering TFGß-1 serving as transformation agent.

Hattersley et al. (WO 96/39170) disclose a two factor composition for inducing cartilaginous tissue formation using a cartilage formation-inducing protein and a cartilage maintenance inducing protein. Specific recombinant cartilage formation inducing protein(s) are specified as BMP-13, MP-52, and

BMP-12, and cartilage maintenance-inducing protein(s) are specified as BMP-9. In one embodiment, BMP-9 is encapsulated in a resorbable polymer system and delivered to coincide with the presence of cartilage formation inducing protein(s).

Laurencin et al., (US-5,629,009) disclose a chondrogenesis-inducing device, consisting of a polyanhydride and polyorthoester, that delivers water soluble proteins derived from demineralized bone matrix, TGFß, EGF, FGF, or PDGF.

The results of the approaches to cartilage repair as cited above are encouraging but they are not satisfactory. In particular, the repair tissue arrived at is not fully hyaline in appearance and/or it does not contain the proper chondrocyte organization. Furthermore, previous approaches to cartilage repair have been addressed to very small defects and have not been able to solve problems associated with repair of large, clinically relevant defects.

One reason that previous approaches failed to adequately repair cartilage may be that they were not able to recapitulate natural cartilage ontogeny faithfully enough, this natural ontogeny being based on a very complicated system of different factors, factor combinations and factor concentrations with temporal and local gradients. A single recombinant growth factor or two recombinant growth factors may lack the inductive complexity to mimic cartilage development to a sufficient degree and/or the delivery systems used may not have been able to mimic the gradient complexity of the natural system to a satisfactory degree.

Previous approaches may also have failed because growth factor concentrations were not able to be maintained over a sufficient amount of time , which would prevent a full and permanent differentiation of precursor cells to chondrocytes. The loss of growth factor could be caused by diffusion, degradation, or by cellular internalization that bypasses the BMP/TGFß receptors. Maintaining a sufficient growth factor concentration becomes particularly important in repair of large sized defects that may take several days or several weeks to fully repopulate with cells.

The object of this invention is to create a composition for improved cartilage repair in vivo. The inventive composition is to enable in vivo formation of repair cartilage tissue which tissue resembles endogenous cartilage (in the case of articular cartilage with its specific chondrocyte spatial organization and superficial, intermediate, and deep cartilage zones) more closely than repair tissue achieved using known compositions for inducing cartilage repair. A further object of the invention is to create a device for cartilage repair which device contains the inventive composition.

This object is achieved by the composition and the device as defined by the claims.

Brief description of the invention

The inventive composition basically consists of a naturally derived osteo-inductive and/or chondroinductive mixture of factors (e.g. derived from bone) or of a synthetic mimic of such a mixture combined with a nanosphere delivery system. A preferred mixture of factors is the combination of factors isolated from bone, known as BP and described by Poser and Benedict (WO 95/13767). The nanosphere delivery system consists of nanospheres defined as polymer particles of less than 1000 nm in diameter (whereby the majority of particles preferably ranges between 200-400 nm) in which nanospheres the combination of factors is encapsulated. The nanospheres are loaded with the mixture of factors in a weight ratio of 0.001 to 17% (w/w), preferably of 1 to 4% (w/w) and have an analytically defined release profile (see description regarding Figure 2) showing an initial burst of 10 to 20% of the total load over the first 24 hours and a long time release of at least 0.1 per day during at least seven following days, preferably of 0.1 to 1% over the following 40 to 60 days. The nanospheres are composed of e.g. (lactic acid - glycolic acid)-copolymers (Poly-(D,L)lactic acid-glycolic acid) made of 20 to 80% lactic acid and 80 to 20% of glycolic acid, more preferably of 50% lactic acid and 50% of glycolic acid.

The loaded nanospheres are e.g. made by phase inversion according to Mathiowitz et al. (Nature, 386: 410-413, 1997) or by other methods known to those skilled in the art (Landry, Ph.D Thesis, Frankfurt, Germany).

The inventive composition is advantageously utilized as a device comprising any biodegradable matrix including collagen type I and II, and hyaluronic acid

in which matrix the nanospheres loaded with the factor combination is contained. The matrix can be in the form of a sponge, membrane, film or gel. The matrix should be easily digestible by migrating cells, should be of a porous nature to enhance cell migration, and/or should be able to completely fill the defect area without any gaps.

It is surprisingly found that the inventive composition consisting of an osteo-inductive and/or chondroinductive combination of factors (e.g. derived from natural tissue) encapsulated in nanospheres as specified above, if applied to a defect area of an articular cartilage, leads to the transformation of virtually all precursor cells recruited to the repair area to chondrocytes, and furthermore, leads to a homogenous chondrocyte population of the repair area and to a chondrocyte order and anisotropic appearance as observed in endogenous hyaline cartilage. These findings encourage the prospect that the inventive composition may lead to significant improvements also regarding repair of large defects.

As mentioned above, instead of an osteoinductive and/or chondroinductive mixture of factors derived from bone (BP), the inventive composition may comprise natural factor mixtures derived from other tissues (e.g. cartilage, tendon, meniscus or ligament) or may even be a synthetic mimic of such a mixture having an osteoinductive and/or chondroinductive effect. Effective mixtures isolated from natural tissue seem to contain a combination of proliferation, differentiation, and spatial organizing proteins which in combination enhance the tissue rebuilding capacity more effectively than single proteins (e.g. recombinant proteins).

The specified, analytically defined release profile of such factor mixtures from nanospheres results in the formation of concentration gradients of proliferation and differentiation factors, which obviously mimics the complex gradients of factors observed during natural development very well. The nanosphere extended release profile is sufficient to provide growth factor during the time frame that repair cells arrive into the matrix. The release profile obviously leads to a homogenous population of a matrix with precursor cells, to full differentiation of virtually all of the precursor cells to chondrocytes, and to the formation of an endogenous hyaline cartilage structure.

Another advantage of the inventive composition is that when the nanospheres are placed in a matrix to form a device for cartilage repair, they are randomly distributed and remain in place when in a joint cartilage defect. During cellular infiltration and differentiation, the nanospheres are in the correct position over the correct time frame.

Nanospheres have been demonstrated to adhere to the gastrointestinal mucus and cellular linings after oral ingestion (Mathiowitz et al., Nature, 386 410-413 1997). We envisage that nanospheres also adhere to cartilage precursor cells and furthermore, may also adhere to BMP/TGFß receptors located on the cell membrane. This property allows localized high-efficiency delivery to the target cells and/or receptors. Because of the nanosphere small size and the chemical properties, they are more effective than liposomes or diffusion controlled delivery systems. The efficient delivery to the receptors will facilitate chondrogenesis.

Derived from the above findings, we envisage the following mechanism for cartilage repair using the effect of the inventive composition. During the first 24 hours (initial burst) 10 to 20% of the total load of the factor mixture is released from the nanospheres into the matrix and diffuses into the synovial environment. Following the initial burst, the nanospheres begin to release factors at a slow rate, which produces gradients of proliferation, differentiation, and spatial organizing proteins. In response to such gradients, precursor cells migrate to the defect site. The loaded nanospheres adhere to cartilage precursor cells and to the BMP and TGFß receptors to provide localized highly efficient delivery. The precursor cells become differentiated to chondrocytes and secrete type II collagen and cartilage-specific proteoglycans. The composition of the present invention stimulates differentiation of virtually all of these cells to overt chondrocytes and induces an ordered cartilage structure which closely resembles hyaline cartilage. Furthermore, we envisage that this release system will allow homogenous repair of large defect sites and repair of defects from patients with low quantities of precursor cells.

For in vivo cartilage repair, the inventive device consisting of a matrix and the loaded nanospheres is placed in a chondral lesion that was caused by trauma, arthritis, congenital, or other origin. The damage can result in holes or crevices or can consist of soft, dying, or sick cartilage tissue that is removed surgically prior to implantation of the device. Because of the unique properties of the inventive device precursor cells populate the matrix, differentiate to chondrocytes, and form hyaline cartilage.

Application of the inventive composition (without matrix) e.g. by injection can be envisaged also, in particular in the case of small defects. Thereby at least 2µg of the composition per ml of defect size is applied or at least 20ng of the

osteoinductive and/or chondroinductive mixture encapsulated in the nanospheres is applied per ml defect size.

The inventive composition and the inventive device are suitable for repair of cartilage tissue in general, in particular for articular cartilage and for meniscus cartilage.

Brief description of the Figures:

The following figures illustrate the physical and chemical parameters of the inventive composition, the in vitro cartilage inductive activity of BP released from nanospheres and in vivo repair of an articular cartilage defect using the inventive device.

Figure 1 shows a scanning electron micrograph of BP-loaded nanospheres;

Figure 2 shows the release profile (cumulative release vs. time) of the inventive composition;

Figure 3 shows the release profile of the inventive composition compared with release profiles of nanosphere delivery systems loaded with other proteins;

Figure 4 shows the volume of a cartilage defect vs. the days required for populating the defect with repair cells;

Figure 5 shows micromass cultures in the presence or absence of nanospheres loaded with BP;

Figure 6 shows cartilage marker analyses for in vitro cultures containing BP only and for similar cultures containing the inventive composition;

Detailed description of the invention:

Figure 1 shows a scanning electron micrograph of BP-loaded nanospheres. The microparticle sizes range from 100-1000 nm with the majority of individual particles ranging between 200-400 nm.

The release rate profile of the inventive composition was determined by in vitro analysis of BP delivered from nanospheres. These nanospheres were made by phase inversion according to the method as disclosed by Mathiowitz et al. (Nature 386, 410-414, 1997) of ((DL)lactic acid / glycolic acid)-copolymer containing the two acids in a weight ratio of 50:50 and they were loaded with 1% and with 4% of BP.

For determination of the release rate profile, the nanospheres were placed in a sterile saline solution and incubated at 37°C. BP released into the supernatant was measured using a BCA assay (Pierce). BP released from the nanospheres as specified shows two successive and distinct profile parts: a fast release (initial burst) of approximately 10 to 20% of the loaded BP over the

first 24 hours and a slow release of 0.1 to 1% per day (cumulative 40% to 50%) over 40 to 60 days (**Fig. 2**).

The release is intermediate between zero-order and first-order kinetics. Both the 1% and 4% encapsulated BP have similar release profiles.

For attaining release rate profiles as specified above and as necessary for the improved results in cartilage repair the nanospheres are to be adapted accordingly when using factor mixtures other than BP. Thereby, e.g the composition of the nanosphere copolymer, the molecular weight of the polymer molecules and/or the loading percentage of the nanospheres may be changed. The optimum nanosphere character for each specific case has to be found experimentally whereby the release rate profile is analyzed in vitro as described above.

In the same way, the nanosphere delivery system can be modified regarding the percentage of BP to be released in the first 24 hours, percentage of BP to be released after 24 hours and/or length of time after the first 24 hours during which the remainder of BP is released. In addition, the percentage of BP loaded to the nanospheres is of course variable too, whereby for obtaining the results as described for the specified composition, all the modifications are to be chosen such that the resulting delivery keeps within the range as specified.

All of the above parameters can be modified to account for the patient's age, sex, diet, defect location, amount of blood present in the defect, and other

clinical factors to provide optimal cartilage repair. For example, nanospheres with longer release rates are used for treating larger defects and/or for patients with fewer precursor cells (e.g. older patients or patients with degenerative symptoms). In contrast, patients with larger quantities of progenitor cells and/ or smaller defects may require a shorter release rate profile.

Figure 3 shows the release profile as shown in Fig. 2 for nanospheres as specified above loaded with BP and with other proteins (same loading percentages) such as BSA (bovine serum albumin) or lysozyme. The drastically different release characteristics shows that the profile is dependent on the protein type also. The same is valid for a more hydrophobic mixture of bovine bone derived proteins (PIBP).

Figure 3 illustrates the singularity of the inventive combination consisting of the specific delivery system (nanospheres as specified above encapsulating the factors) and the specific protein mixture (BP) which is obviously the key to the improved results in cartilage repair as observed when using the inventive composition or device.

To determine the length of time required for precursor cell repopulation of different sized defects, the following calculation was performed. We estimate that approximately 50,000 cells are recruited to the defect/day. Since the cellular density of cartilage is about 4×10^7 cells/ml, a 10 µl volume defect will take approximately 8 days to fill with cells. **Figure 4** plots the number of days required to fill different volume defects with cells. The Figure assumes an infinite supply of cells and a constant rate of cell attraction to the defect site. The graph demonstrates that the larger a defect size is, the more time is

required to completely fill it with cells. Since a 60 μl volume defect will take over 45 days to fill, this Figure demonstrates the necessity for a long term release of factors to induce differentiation of the precursor cells over up to a two month period.

To determine whether BP bioactivity is harmed by the encapsulation process and to determine whether the released BP was fully bioactive, the following assay was performed. Previously, it was demonstrated that 10T1/2 micromass cultures exposed to BP induce formation of a three dimensional spheroid structure that can be observed macroscopically in tissue culture wells (Atkinson et al., J. Cellular Biochem. 65: 325-339, 1997). BP concentrations equal or greater than 20 ng/ml were required for spheroid formation. No spheroid forms in the absence of BP or at concentrations less than 10 ng/ml (see following table). In this assay, 10T1/2 mesenchymal stem cells act as in vitro models for the precursor cells recruited to a natural defect.

We employed the same assay to test the bioactivity of BP released from 1% loaded nanospheres. BP was eluted from nanospheres at 37°C in a 5% CO_2 humidified incubator. After 24 hours 16% BP is released; and between 24 hours and 7 days, 7% BP was released (Fig. 2). The supernatant was collected, serial dilutions were made, and the supernatant was added to 10T1/2 micromass cultures. BP released from nanospheres at both time points formed spheroids at concentrations greater than 20 ng/ml, but not at concentrations between 0 and 10 ng/ml (see following table). Non-encapsulated BP also formed spheroids at concentrations greater than 20 ng/ml, but not at concentrations between 0 and 10 ng/ml. We conclude that both nanosphere encapsulation and release of BP does not inhibit BP bioactivity.

Spheroid formation (- = no spheroid formation; + = spheroid formation):

state of used BP	BP concentration (ng/ml) 0 - 10	20 - 1000
non-encapsulated BP	-	+
released from nanospheres (24 h)	-	+
released from nanospheres (168 h)	-	+

To determine the effect of BP slow release in the direct presence of micromass cultures, the following assay was performed. Nanospheres were washed for 24 hours and the supernatant was discarded. The nanospheres were then added to micromass cultures at a quantity such that 10 or 25 ng/ml of BP would be released over 24 hours. Release of 25 ng/ml resulted in spheroid formation whereas release of 10 ng/ml did not form spheroids (**Fig. 5**). Similarly, the addition of 10 ng of non-encapsulated BP per ml did not form a spheroid whereas the addition of 25 ng of non-encapsulated BP per ml did form a spheroid. Regarding the specific in vitro set -up, we conclude that slow release of BP over 24 hours is as effective as a single dose of BP.

To determine whether the BP released from nanospheres was as chondrogenic as non-encapsulated BP, spheroids were analyzed for type II collagen and proteoglycan content. 10T1/2 spheroids from the above assay that had formed with 1 µg of released BP per ml or 1 µg of non-encapsulated BP per ml were tested histologically with Azure and H+E stains and immunocytochemically with antibodies to type II collagen after 7 days. Both encapsulated and non-encapsulated BP induced cartilage markers such as type II collagen, proteoglycan, and round cell shape (**Fig. 6**). In addition, no qualitative differences were observed between encapsulated and non-encapsulated BP with respect to

cell quantity, viability, morphology, or organization (Fig. 6). We conclude that BP retains full chondrogenic capacity after release from nanospheres.

The in vitro models used for determining the chondroinductive effect of BP differ from the in vivo case by the fact that in the in vitro case the precursor cells are present in an appropriate number and in an appropriate distribution whereas in the in vivo case the precursor cells first have to populate the defect and for this reason have to migrate into the defect. Only in the latter case and for achieving repair cartilage which resembles natural cartilage to a high degree, it is essential for the BP to be released over a prolonged time period according to a specific release profile.

EXAMPLE

The following example shows that BP released from nanospheres induces cartilage repair in chondral defects in vivo whereby virtually all cells recruited to the defect become chondrocytes, whereby the cell structure obtained is ordered, and whereby a hyaline matrix is built up.

Using a sheep model, unilateral defects of 0.5mm width, 0.5mm depth and 8 to 10mm length were created in the trochlear groove of the patella. The defects did not penetrate the subchondral bone. The sheep employed in this study were seven years old and displayed degenerative symptoms, including brittle bones, chondromalacia, and subchondral cysts. Because of their advanced age and degenerative symptoms, these amimals probably have decreased numbers of precursor cells. The defects were then dressed

according to Hunziker and Rosenberg (J. Bone Joint Surg. 78A(5): 721-733, 1996) with minor changes. Briefly, after enzymatic proteoglycan removal with Chondroitinase AC, 2.5µl of a solution containig 200 units Thrombin per ml was placed in the defect. Then, a paste was filled into the defect, the paste containing per ml: 60mg Sheep Fibrinogen (Sigma), 88mg Gelfoam (Upjohn) and either 10µg of BP-nanospheres or 10µg of BP-nanospheres plus 80ng rhIGF-1 (R+D Systems).

The nanospheres used were the nanospheres as specified in the description regarding Figure 2 and they were loaded with 1% (w/w) of BP.

Assuming that the in vitro determined release rate is approximately the same as for the in vivo case, 10 to 20ng BP per ml were released during the first 24 hours and approximately 0.1 to 1ng per day for the following approximately 60 days.

After eight weeks, necropsies were performed. The repaired cartilage histology showed that virtually all of the precursor cells were differentiated to chondrocytes throughout the defect. In addition, there was an ordered cartilage appearance with cells on the top being more flattened morphologically than cells in the center and with the presence of ordered, stacked chondrocytes in the lowest zone. The repaired cartilage was fully integrated into the endogenous tissue. In addition, the cartilage repaired with only BP-nanospheres was not significantly different from the cartilage repaired using BP-nanospheres plus IGF-1.

In conclusion, these results demonstrate that BP released from nanospheres is sufficient for cartilage repair and that no addintional factor is required (such as e.g recombinant factor IGF-1). Using the inventive device constitutes a one step method for cartilage repair, whereby the nanosphere release of BP is sufficient for differentiation of virtually all of the precursor cells to chondrocytes and for induction of an ordered cartilage structure.

Other Publications:

Archer CW, Morrison EH, Bayliss MT, Ferguson MW: The development of articular cartilage: II. The spatial and temporal patterns of glycosaminoglycans and small leucine-rich proteoglycans; J Anat (ENGLAND) 189 (Pt 1): 23-35 (1996)

Atkinson BL, Fantle, KS, Benedict JJ, Huffer WE, Gutierrez-Hartmann A: A Combination of Osteoinductive Bone Proteins Differentiates Mesenchymal C3H/10T1/2 Cells Specifically to the Cartilage Lineage; J. Cellular Biochem. 65: 325-339 (1997).

Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM: Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone; Proc Natl Acad Sci U S A, Dec, 87(24): 9843-7 (1990)

Denker AE, Nicoll SB, Tuan RS: Formation of cartilage-like spheroids by micromass cultures of murine C3H10T1/2 cellls upon treatment with transforming growth factor ß1'; Differentiation 59(1): 25-34 (1995)

Denker AE, Nicoll SB, Tuan RS: 41st Annual Meeting Orthop. Res. Society.(abstract): 465 (1995)

Flechtenmacher J, Huch K, Thonar EJ, Mollenhauer JA, Davies SR, Schmid TM, Puhl W, Sampath TK, Aydelotte MB, Kuettner KE: Recombinant human osteogenic protein 1 is a potent stimulator of the synthesis of cartilage proteoglycans and collagens by human articular chondrocytes; Arthritis Rheum, Nov, 39(11): 1896-904 (1996)

Hunziker EB and Rosenberg LC: Repair of Partial-Thickness Defects in Articular Cartilage: Cell Recruitment from the Synovial Membrane; J. Bone Joint Surgery 78-A(5): 721-733 (1996)

Kim S, Turker MS, Chi EY, Sela S, Martin GM: Preparation of multivesicular liposomes; Bioch. et Biophys. Acta 728:339-348 (1983)

Landry FB: Degradation of Poly (D,L-lactic acid) Nanoparticles in artificial gastric and intestinal fluids; in vivo uptake of the nanoparticles and their degradation products; Thesis for the Dept. of Biochemistry, Pharmacy, and Food Chemistry of the Johann Wolfgang Goethe University in Frankfurt, Germany

Luyten FP, Cunningham NS, Ma S, Muthukumaran N, Hammonds RG, Nevins WB, Woods WI, Reddi AH: Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation; J Biol Chem, 264(23): 13377-80 (1989)

Massague J: TGFß Signaling: Receptors, Transducer, and Mad Proteins; Cell 85: 947-950 (1996)

Mathiowitz E, Jacob JS, Jong YS, Carino GP, Chickering DE, Chaturvedi P, Santos CA, Vijayaraghavan K, Montgomery S, Bassett M, Morrell C: Biologically erodable microspheres as potential oral drug delivery systems; Nature 386: 410-4 (1997)

Morrison EH, Ferguson MW, Bayliss MT, Archer CW: The development of articular cartilage: I. The spatial and temporal patterns of collagen types; J Anat (ENGLAND) 189(Pt 1): 9-22 (1996)

Mow VC, Ratcliff A, Poole AR: Cartilage and diarthrodial joints as paradigms for hierarchical materials and stuctures; Biomaterials 13(2): 67-97 (1992)

Sailor LZ, Hewick RM, Morris EA: Recombinant human bone morphogenetic Protein-2 maintains the articular chondrocyte phenotype in long-term culture; J. Orthop. Res. 14: 937-945 (1996)

Urist MR: Bone: formation by autoinduction; Science 150: 893-899 (1965)

Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, LaPan P, Luxenberg DP, McQuaid D, Moutsatsos I, Nove J, Wozney JM: Recombinant human bone morphogenetic protein induces bone formation;' Proc Natl Acad Sci U S A, 87(6): 2220-4 (1990)

Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA: Novel Regulators of bone formation: molecular clones and activities; Science 242: 1528-34 (1988)

5

CLAIMS

1. Composition for inducing in vivo cartilage repair comprising an osteoinductive and/or chondroinductive mixture of factors derived from natural tissue or a sythetic mimic of such a mixture encapsulated in nanospheres, whereby the nanospheres are polymer particles having a size of less than 1000 nm and an in vitro analytically determined release rate profile with an initial burst of 10 to 20% of the total load over the first 24 hours and a long time release of at least 0.1 % per day during at least seven following days and whereby the nanospheres are loaded with between 0.001 and 17% weight percent of the mixture of factors.

2. Composition according to claim 1, **characterized** in that the long term release is between 0.1 and 1 % of the total load per day over between 40 and 70 days.

3. Composition according to claim 1 or 2, **characterized** in that the osteoinductive and/or chondroinductive mixture of factors is derived from bone, cartilage, tendon, meniscus or ligament.

4. Composition according to claim 1 or 2, **characterized** in that the osteoinductive and/or chondroinductive mixture of factors is the mixture known as BP (bone protein) derived from bovine long bones and partly purified.

5. Composition according to claim 4, **characterized**, in that the nanospheres are loaded with between 1 and 4% weight percent of BP.

6. Composition according to one of claims 1 to 5, **characterized** in that the nanospheres consist of ((D,L)lactic acid / glycolic acid)-copolymer containing 20 to 80% of lactic acid and 80 to 20% of glycolic acid.

7. Composition according to one of claims 1 to 5, **characterized** in that the ((D,L)lactic acid / glycolic acid)-copolymer contains 50% of lactic acid and 50% of glycolic acid.

8. Composition according to one of claims 1 to 7, **characterized** in that the nanospheres are made by phase inversion.

9. Device containing the composition according to one of claims 1 to 8 and further comprising a porous biodegradable matrix.

10. Device according to claim 9, **characterized** in that it contains at least 2μg of loaded nanospheres per ml.

11. Device according to claim 9, **characterized** in that it contains at least 20ng of the osteoinductive and/or chondroinductive mixture of factors per ml.

12. Device according to one of claims 9 to 11, **characterized** in that the matrix has the form of a sponge, membrane, film or gel.

13. Device according to one of claims 9 to 12, **characterized** in that the matrix consists of collagen type I, collagen type II or hyaluronic acid.

14. Use of the composition according to one of claims 1 to 8 for preparing a device for in vivo cartilage repair.

15. Use according to claim 14, **characterized** in that the cartilage is articular cartilage or meniscus cartilage.

16. Use of the composition according to one of claims 1 to 8 for cartilage repair on an animal with a degenerative disease.

17. Use of the device according to one of claims 9 to 13 for cartilage repair on an animal with a degenerative disease.

18. Method for in vivo cartilage repair comprising the step of filling a cartilage defect with a device according to one of claims 9 to 13.

19. Method according to claim 18, **characterized** in that the defect is dressed before filling.

20. Method for in vitro cartilage repair comprising the step of applying to the cartilage defect a composition according to one of claims 1 to 8.

21. Method according to claim 20, **characterized** in that the composition is applied by injection.

22. Method according to claim 20 or 21, **characterized** in that the composition is applied in an amount of at least 2μg per ml defect size.

23. Method according to claim 20 or 21, **characterized** in that the composition is applied in an amount such that the osteoinductive and/or chondroinductive mixture is present in the defect in an amount of at least 20ng per ml defect size.

FIGURE 1

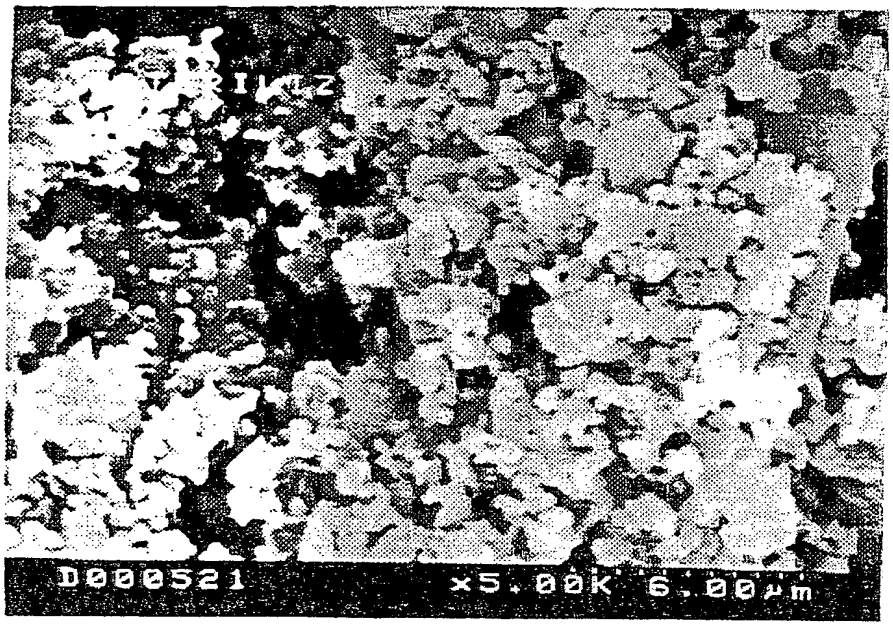


FIGURE 2

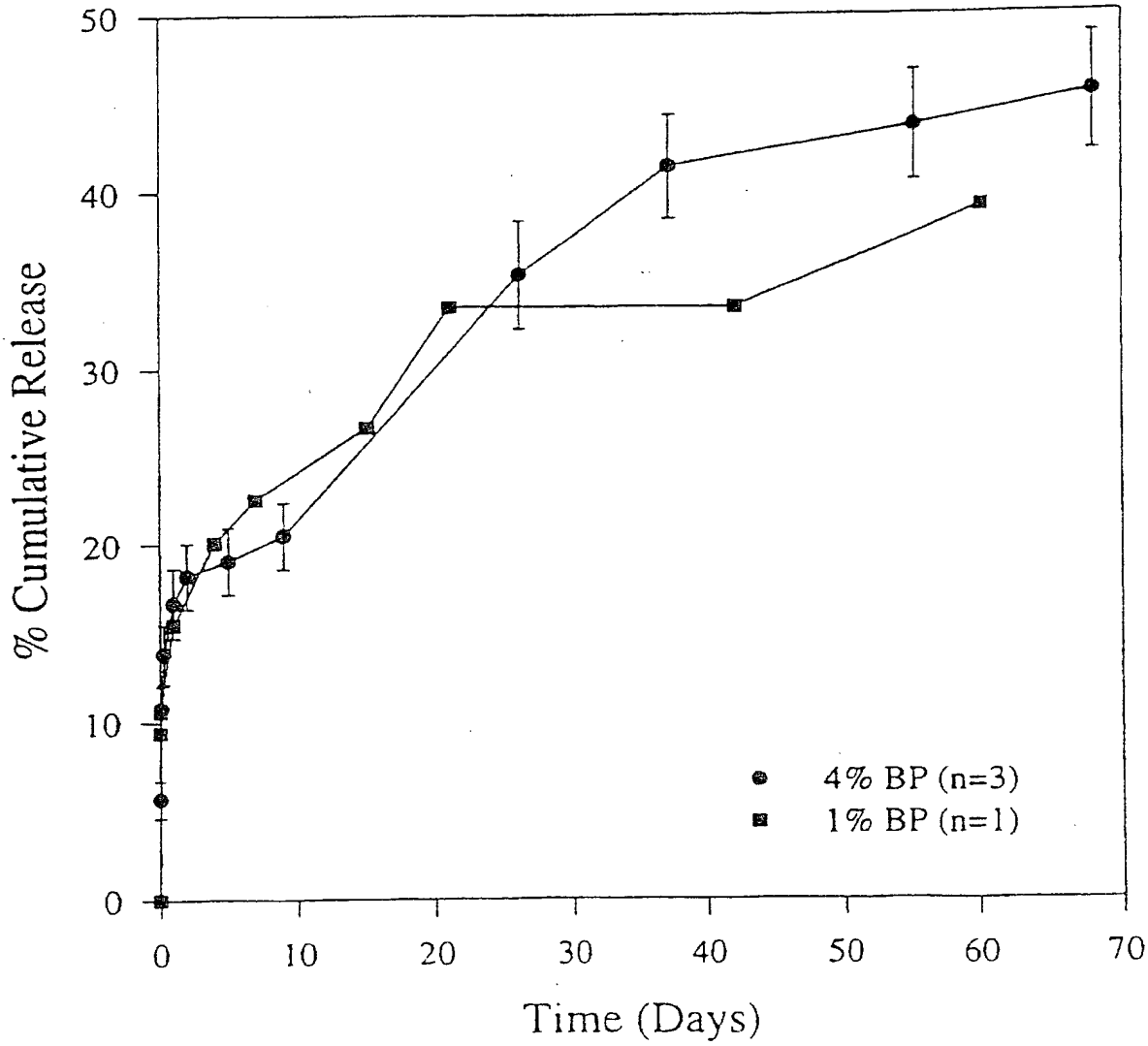


FIGURE 3

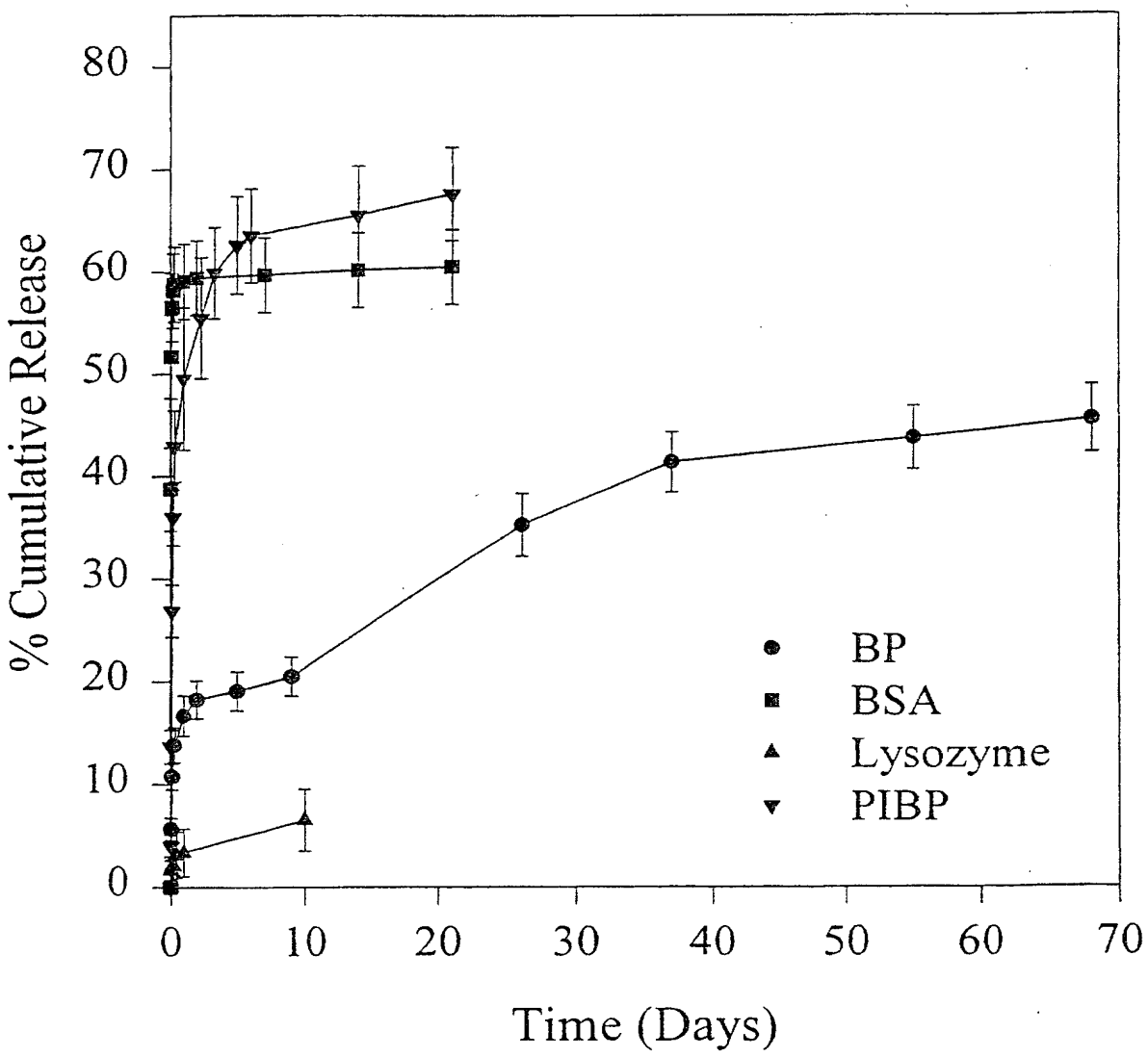


FIGURE 4

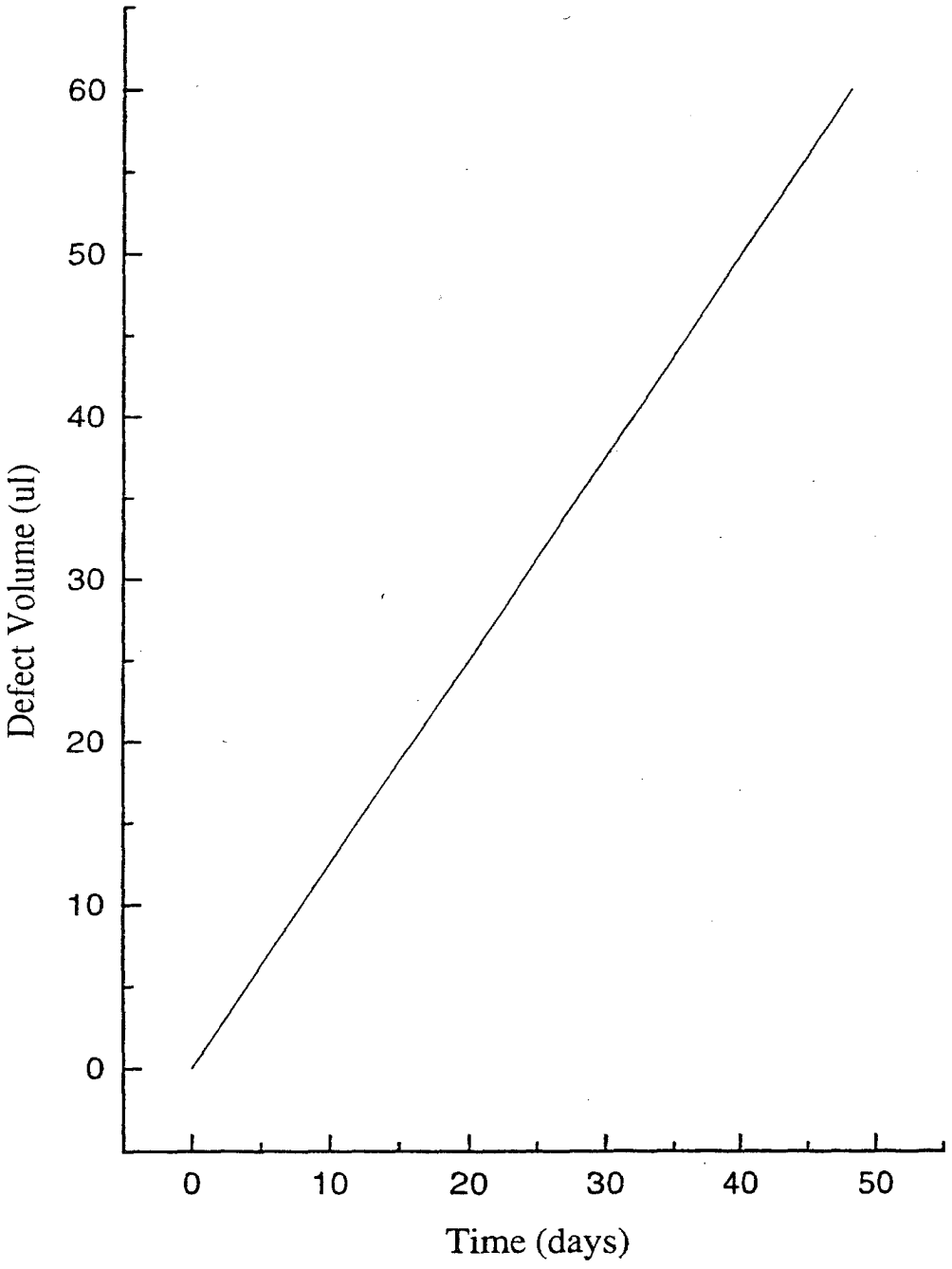


FIGURE 5

- BP + BP Nanospheres

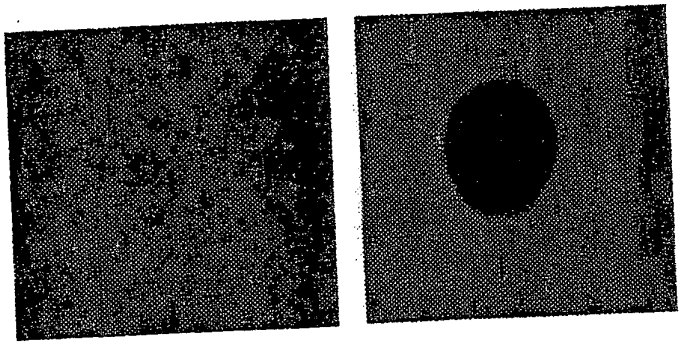
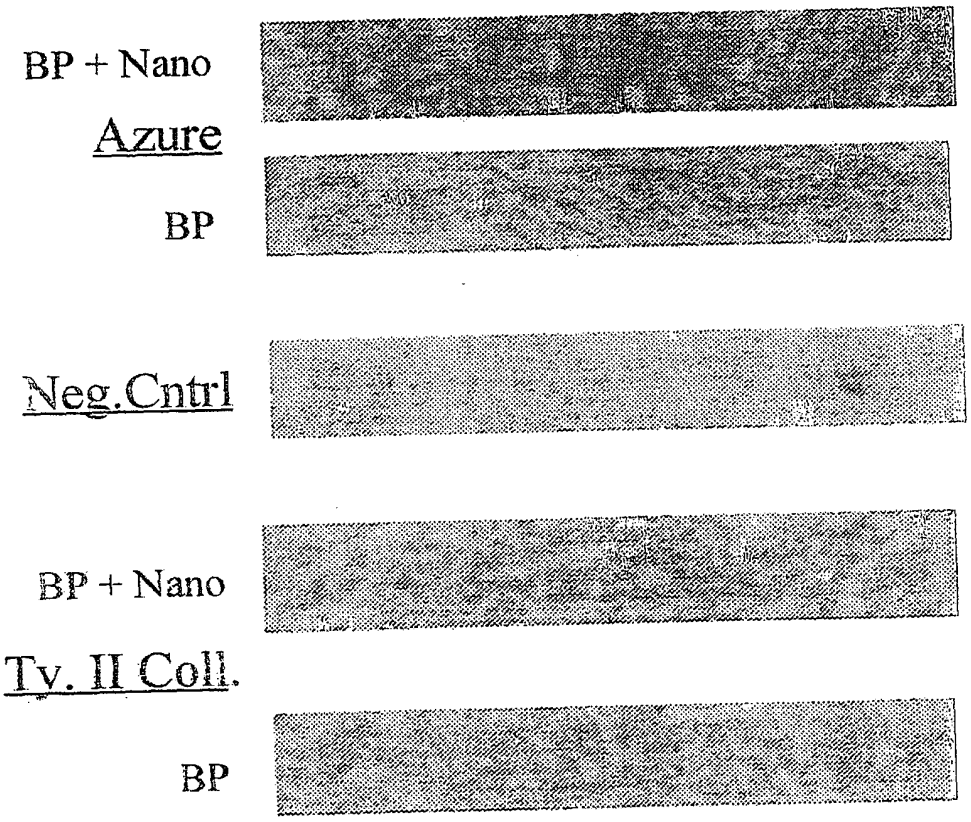


FIGURE 6



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61K9/51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SONG C X ET AL: "FORMULATION AND CHARACTERIZATION OF BIODEGRADABLE NANOPARTICLES FOR INTRAVASCULAR LOCAL DRUG DELIVERY" JOURNAL OF CONTROLLED RELEASE, vol. 43, no. 2/03, 18 January 1997, pages 197-212, XP000632668 see abstract see page 203, right-hand column, line 3 - line 10; figure 5; table 3 see page 207, left-hand column, paragraph 2 - right-hand column, paragraph 1; figure 10 --- -/--	1-4,6-9, 12-15, 18-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 1999	22. 01. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heck, G

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

I.. .national Application No
PCT/EP 98/05100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 368 858 A (HUNZIKER ERNST B) 29 November 1994 see claims 1-6,8 see column 5, line 59 - column 6, line 34 see column 7, line 26 - column 8, line 6 see column 8, line 35 - line 50	1-4,6-9, 12-15, 18-21
A	US 4 620 327 A (CAPLAN ARNOLD I ET AL) 4 November 1986 see abstract see column 1, line 59 - column 2, line 3 see claim 1	4,5
A	US 5 595 722 A (GRAINGER DAVID J ET AL) 21 January 1997 see column 17, line 48 - column 18, line 33	8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/ 05100

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 18 and 19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 18 and 19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. (Rule 39.1(iv) PCT)

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/05100

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5368858 A	29-11-1994	US 5206023 A	27-04-1993
		AT 121943 T	15-05-1995
		AU 667032 B	07-03-1996
		AU 1412892 A	07-09-1992
		CA 2101556 A	01-08-1992
		CN 1064813 A	30-09-1992
		DE 69202332 D	08-06-1995
		DE 69202332 T	04-01-1996
		DK 569541 T	10-07-1995
		EP 0569541 A	18-11-1993
		ES 2072144 T	01-07-1995
		IE 67515 B	03-04-1996
		IL 100799 A	16-10-1996
		JP 6505258 T	16-06-1994
		NZ 260125 A	27-07-1997
		WO 9213565 A	20-08-1992
US 4620327 A	04-11-1986	AU 4601485 A	10-02-1986
		EP 0188552 A	30-07-1986
		WO 8600526 A	30-01-1986
US 5595722 A	21-01-1997	US 5847007 A	08-12-1998
		AU 6277396 A	30-12-1996
		CA 2223595 A	19-12-1996
		EP 0833624 A	08-04-1998
		WO 9640098 A	19-12-1996
		US 5545569 A	13-08-1996
		US 5770609 A	23-06-1998
		CA 2162586 A	24-11-1994
		EP 0710116 A	08-05-1996
		JP 8510451 T	05-11-1996
		WO 9426303 A	24-11-1998
		US 5472985 A	05-12-1995
		US 5599844 A	04-02-1997
		US 5773479 A	30-06-1998
		CA 2162587 A	24-11-1994
		EP 0702557 A	27-03-1996
		JP 8510209 T	29-10-1996
		WO 9426291 A	24-11-1994
		US 5811447 A	22-09-1998
		US 5733925 A	31-03-1998
		CA 2154698 A	04-08-1994
		EP 0681475 A	15-11-1995
		JP 8506112 T	02-07-1996
		WO 9416706 A	04-08-1994